# ENTEROTOXIC STAPHYLOCOCCI IN UPPER RESPIRATORY TRACT DISEASES

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- Poland -



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By:

U. S. DEPARTMENT OF COMMERCE
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# ENTEROTOXIC STAPHYLOCOCCI IN UPPER RESPIRATORY TRACT DISEASES

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/Following is a translation of an article by Krystyna Radomanska, of the Microbiology Institute of the Pharmacology Division of the Lublin Academy of Medicine (Director: Dr biol. Wodzimierz Nicewicz), published in the Polish-language periodical Polski Tygodnik Lekarski (Polish Medical Weekly), Vol 16, No 18, Warsaw, 1961, pages 671-673./

The purpose of this work was the study of Staphylococcus aureus and St. albus, and the indication of the characteristics of the individual strains which make possible their listing among the pathogenic microbes. Staphylococcus strains were used which were isolated from persons with pharyngeal and nasal diseases; they were examined by means of a biological reaction on frogs, with special regard to enterotoxins. It is generally assumed (among others by BURBIANKA and coworkers) that only the cats are suitable for the demonstration of enterotoxins. According to LACHOWICZ, the determination of enterotoxins on cats is more authentic than on frogs which give less definite results. Due to the difficulty of procurement of a large number of cats, this work was limited to the test on frogs whose suitability was confirmed by ROBINTON and PISU.

## Personal Researches

Procedure -- Smears were collected from the nasal and pharyngeal cavity of sick people (exclusively adults) who came to the laryngological outpatient department of the Medical Academy in whom a disease was found on the basis of an infection of the pharyngeal and nasal cavity such as inflammation of the maxillary sinuses, inflammation of the tonsils, inflammation of the larynx and abscesses and infiltrations.

The collected material was inoculated on agar plates by the method of dilution, maintaining the distinction of the smear of nasal and pharyngeal origin. The isolated colonies of staphylococci were transferred to agar slant. All strains were examined for the property of causing haemolysis, producing coagulase and phosphatase, decomposition of sugars, and casein curdling in milk. The agglutination titre with non-specific serum

(horse serum) and the formation of enterotoxin was also determined.

The haemolysis was determined by cultivating the tested strains on blood agar (lamb blood) in the thermostat for 24 hours, and then keeping it in cold storage until the moment of reading the results.

The ability of coagulase formation was examined by the test-tube method. To 0.5 ml of fivefold diluted human plasma a few drops of staphylococcic bouillon culture are added. After three hours' incubation period, the results are observed the next day.

The ability of phosphatase production was examined by adding a few drops of diluted NaOH to the liquid culture of staphylococci which contains 0.01 percent. of the sodium salt of phenolphthaleino-phosphoric acid. The appearance of red coloration showed the presence of phosphatase-producing staphylococcus strain.

For the confirmation of the fermentative properties, the examined material was cultivated on pepton water with a colored indicator, Neutral Red, with addition of 1 percent. mannite, galactose, saccharose and maltose, and it was kept in thermostat for 24 hours, and then the appearance of acids and gases was diagnosed.

For the corroboration of the casein-curdling property in milk, examination was made on fresh, skimmed milk which, after being poured into test tubes, was three times sterilized in Koch's apparatus for 15 minutes. After inoculation with staphylococci, this medium was kept three days in the thermostat.

The non-specific agglutination reaction was made according to the test tube method with horse serum. For this purpose, increasing dilution titres of the serum were prepared (from 1:20 to 1:640) to which 0.5 ml bacterial suspension was added (one loop of constant culture to 5 ml of physiological saline). The blank test tube contained only 0.85% NaCl solution with addition of suspension. After mixing the contents of the test tubes they were kept in the thermostat for 3 hours, and then at room temperature, while the results were read after 24 hours. In this reaction, an attempt was made to determine an agglutination titre which in case of pathogenic strains should not exceed the values of 1:40 - 1:80.

For the confirmation of the enterotoxin-forming property in the examined strains, the following was done: -- the 24-hour staphylococcic culture (on sugar bouillon) was taken in an amount of about 10 ml, and it was inoculated on a semi-liquid medium (0.3% agar with the addition of 5% starch) in a thin layer in Roux bottles. After the obtaining of a visible growth, $CO_2$ (to 1/3 of the capacity) was introduced to the interior of the bottle, and its outlet was tightly closed at once; then

it was cultivated in thermostat for 3-4 days. Later, the whole contents of the container was drained through a Seitz filter, after previous slight squeezing through a piece of gauze. The filtrate was boiled for half an hour on water bath and for further examinations it was kept at room temperature.

The biological reactions were made in the months of the spring and the summer with regard to the more intensive reaction of the frogs in this period of time to the administered enterotoxin (PISU). For the experiments, large frogs were used which were caught on wet meadows.

Each filtrate, after previous warming in the thermostat for half an hour, was administered to two frogs simultaneously -- to one with the aid of a pipette in the amount of 0.5 ml directly into the digestive tract, to the other intraperitoneally in the amount of 0.3 ml. The control frogs received physiological saline at the same doses and in double doses. After the administration of the tested material, the frogs were kept under close observation for 6 hours. A full set of symptoms, most often occurring in a definite sequence, was considered a positive reaction:-- contraction and extension of the gastroconemius muscle, opening and closing of the mouth with simultaneous movements resembling marked gulping, rubbing the mouth or the leg against the edge of the container, staring and covering the eyeballs alternately, and the most characteristic phenomenon which has been accepted as the essence of the reaction -- rubbing the mouth with the frontal paws (e.g., with the left paw outwardly, and then pushing in the belly with the right rear paw, then anew with the right front paw rubbing the mouth, and with the left rear paw pushing in the abdomen). This action is rhythmically repeated a few times or more. These phenomena were observed between the first and the third hour after the administration of the filtrate. Only in a single case did these symptoms occur five hours after the administration of the filtrate. The control frogs never showed this complex of symptoms; neither did the frogs which were kept in the vivarium before their use for the experiments.

For the purpose of finding out whether the occurring phenomena are really the result of the enterotoxic action of the tested strain, a known enterotoxic strain was used for control. This strain was cultivated under the same conditions, and in the same manner it was used on a certain group of frogs. Both by intraperitoneal and by peroral administration, the complex of all symptoms occurred which were accepted as conclusive, moreover in many individual frogs a somewhat earlier and more active occurrence of the reaction could be observed.

## Results of Examinations

A total of one hundred staphylococcic strains were isolated:-- 53

strains of St. aureus (among them 22 strains from the pharynx and 31 strains from the nose), and 47 St. albus strains (among them 12 from the pharynx and 35 from the nose). These strains were isolated from 69 smears. Frequently, St. aureus and St. albus occurred in the same smear.

TABLE 1. Characteristics of All Isolated Strains

| Reaction | Isolated staphylococci and their reaction | | | |
|---|---|---|---|---|
| | St. aureus | | St. albus | |
| | + | - | + | - |
| Total number of strains | 53 | | 47 | |
| Haemolysis | 43 | 10 | 2 | 45 |
| Coagulation | 39 | 14 | 1 | 46 |
| Phosphatase | 45 | 8 | 1 | 46 |
| Milk | 11 | 42 | 23 | 24 |
| Agglutination with horse serum at titre 1:80 | 44 | | 22 | |
| " above 1:80 | 9 | | 25 | |
| SUGAR: | | | | |
| mannite | 50 | 3 | 14 | 33 |
| galactose | 52 | 1 | 38 | 9 |
| saccharose | 53 | - | 40 | 7 |
| maltose | 53 | - | 46 | 1 |

The basis of pathogenecity of the staphylococci is admittedly their ability to produce coagulase and phosphatase and to destroy mannite (PAKULA et al.) which properties usually occure together.

Among the 53 aureus strains, 39 were coagulase positive and 45 were phosphatase positive. Only one of the 47 albus strains gave a positive test for coagulase and phosphatase (Table 1).

Out of the 39 coagulase-positive staph. aureus strains, six were non-hemolytic. With regard to the same number of aureus strains of coagulational properties, 31 strains agglutinated with horse serum at a titre not exceeding 1:80.

One of two hemolytic St. albus strains coagulated the plasma, and

showed an agglutination titre of 1:80.

Eleven of the 39 coagulase-positive aureus strains curdled casein in milk. One half out of the coagulase-negative albus strains curdled casein, which shows that -- based upon the observed cases -- this property is rather a mark of the non-pathogenic strains.

TABLE 2. Characteristics of Nine Enterotoxic Strains

| No of strains | Color | Haemolysis | Coagulase | Phosphatase | Milk | Agglutination by horse serum | SUGARS | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Mannite | Galactose | Saccharose | |
| 7 | alb. | # | - | - | - | ## | # | - | - | |
| 41 | aur. | - | # | # | - | # | - | # | # | |
| 44 | aur. | # | # | # | - | # | # | # | # | |
| 62a | aur. | - | # | # | - | ## | # | # | # | |
| 75a | aur. | # | - | # | - | # | # | # | # | |
| 82 | alb. | - | - | - | # | # | - | # | # | |
| 83 | aur. | # | # | # | - | # | # | # | # | |
| 91 | aur. | # | # | # | - | # | # | # | # | |
| 95a | aur. | # | # | # | - | # | # | # | # | |

REMARK: ## means a titre higher than 1:80.

The splitting of mannite basically coincides with the coagulase formation in the St. aureus, whereas in the albus strains which are coagulase-negative this reaction is not typical. The advantage of cultivating on galactose and saccharose is questionable, and the culture on maltose has no practical importance because this sugar is split by almost all the tested strains.

The property of enterotoxin production was confirmed in 7 aureus and 2 albus strains (Table 2). LACHOWICZ found in his work that frogs are less sensitive to enterotoxin (4 strains were positive for cats and frogs, and only 2 for cats); therefore, the positive reactions on frogs obtained in this work (i.e., the present article) can be considered as certain. If these tests would have been carried out simultaneously on cats and frogs, the number of enterotoxic strains could be merely increased.

## Conclusions

1. Enterotoxic strains do not show characteristic biochemical properties which would make possible their differentiation from those strains that do not produce enterotoxin.

2. Enterotoxins can be produced not only by the aureus strains but also by staphylococci classified as albus strains.

3. For the detection of the staphylococcic enterotoxin, a biological reaction can be made also on frogs with positive result.

4. The presence of enterotoxic strains in non-hospitalized carriers of diseases of the upper respiratory pathways can be a source of staphylococcic intoxications.

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Rec'd: 1 July 1960; address: Microbiological Institute, Pharmacy Division, ul. Staszica 4, Lublin.

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